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Megaloblastic Anemia

Olaniyi John Ayodele

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<http://dx.doi.org/10.5772/intechopen.70063>

Abstract

Megaloblastic anemia is a multisystem disorder, which can easily be diagnosed with high index of suspicion and by correct application of its pathogenetic mechanisms. Any factor inhibiting deoxyribonucleic acid (DNA) synthesis, drugs (medications), infections like human immunodeficiency virus (HIV) and gas like nitrous oxide will cause megaloblastosis. However, poor diet, problems with absorption, transportation and metabolism of the vitamins, as well as factors that increase demand and ultimately exhaust the store of the vitamins like chronic hemolytic states, pregnancy, malignancies happen to be the commonest causes of megaloblastic anemia. A complete blood count, blood and marrow films review reflect the typical pathognomonic cytologic appearance of megaloblastic anemia. Logically selected biochemical tests help in establishing diagnosis through determination of serum levels of both folate and cobalamin and assessment of the metabolites, which are considered to be more sensitive and specific. Also, full endoscopic studies are required to confirm the presence of disorders of gastrointestinal tract responsible for impaired absorption. Clinical features are subtle and widely varied. It is highly amenable to therapy once the primary cause is established and managed. Appropriate replacement therapy of deficient nutrient, cobalamin or folate or both, easily corrects anemia. Pernicious anemia often requires lifelong therapy with parenteral cobalamin.

Keywords: anemia, megaloblast, blood and marrow smears review, neuropathies, replacement therapy

1. Introduction

Anemia, technically, describes a condition in which an individual's hemoglobin level (or hematocrit) falls two standard deviations below the average mean of normal for individuals of same age, sex, and altitude [1, 2]. The functional consequence of anemia decreased oxygen carrying capacity of the blood and general tissue hypoxia.

Megaloblastic anemia refers to a group of anemias that have in common a selective reduction in the rate of deoxyribonucleic acid (DNA) synthesis; however, transcription, translation, and protein synthesis proceed normally. Consequently, a resultant unbalanced cell growth ensues and the dichotomy between the rates of cytoplasmic and nuclear maturation widens with each division during erythropoiesis until eventually the cell either dies or omits terminal division making it to survive as oversized end stage cell (macrocytes) with a shortened lifespan. Therefore, the retarded DNA synthesis leads to accumulation of dead and dying megaloblasts in the marrow, creating a spurious appearance of marrow hyperplasia but with a gradual reduction in the number of matured cells being pushed out and eventually progressed to pancytopenia. The megaloblastic anemias are caused by vitamin B₁₂ deficiency, folate deficiency, or by related conditions that caused impaired DNA synthesis.

1.1. History

Many researchers sequentially contributed to the discovery and identification of its etiology. Addison in 1849 was the first to characterize it as anemia, general languor, and debility [3]. In 1877, Osler and Gardner discovered its association with neuropathy and its association with myelopathy was documented 10 years later by Lichtheim. Megaloblasts were identified by Ehrlich in 1880 while the abnormalities in leukocytes were described in 1920. It was confirmed by Minot and Murphy that the disease is reversible by the intake of large amount of liver [4]. Castle, in 1929, discovered the presence of “intrinsic factor” in gastric acid that facilitates the absorption of the “extrinsic factor” [5]. The structure of vitamin B₁₂ was later identified by Hodgkin and this earned him a Nobel Prize [6]. However, it was Herbert, in 1948, who discovered the structure of folic acid and described its link with the causation of megaloblastic anemia [7].

2. Epidemiology

Epidemiological studies on megaloblastic anemia in Nigeria and in Africa are sparse. However, the frequency of megaloblastosis is highest in countries in which malnutrition is rampant and routine vitamin supplementation for elderly individuals and pregnant woman is not available. Faulty preparations of foods and increased demand for folate during pregnancy are the most common causes of megaloblastic anemias.

About 1 in 7500 people develops pernicious anemia in the US per year but this has been modified by current fortification of foods and vitamin supplementations in elderly patients in the US. International statistics showed that pernicious anemia and folate deficiency usually occur in individuals older than 40 years and the prevalence increases with older populations. The incidence of pernicious anemia is reported to be higher in Sweden, Denmark, and United Kingdom than in other developed countries [8].

3. Physiology of cobalamin and folate

Vitamin B₁₂ consists of a corrin ring with a cobalt atom in its center attached to a nucleotide portion and they are termed cobalamins. The biologically inactive pharmacologic preparations

of vitamin B₁₂ include cyanocobalamin and hydroxocobalamin whereas adenosylcobalamin and methylcobalamin that are generated through enzymatic synthesis are the biologically active forms, whereas adenosyl-cobalamin is the tissue form of vitamin B₁₂, methylcobalamin circulates in blood. Although a normal diet provides a large excess of vitamin B₁₂, the daily requirement is about 1–2 µg in adults. In the process of digestion, R-protein, either of salivary or parietal cells origin, binds to liberated cobalamin from complex dietary protein through the action of gastric secretion made up of pepsin and hydrochloric acid. The cobalamin-R protein complex is degraded pancreatic secretions in the duodenum to release free cobalamin that then binds to intrinsic factor that was secreted in the stomach. The cobalamin-intrinsic factor complex is now transported to the terminal ileum where absorption takes place. Failure of physiological activity at any of these points results in megaloblastic anemia. Following absorption, the released vitamin binds a transport protein called transcobalamin (TCII), which transports the vitamin to enterohepatic circulation. Vitamin B₁₂ is stored primarily in the liver in an amount of 2–3 mg, which is 1000-fold in excess of daily requirement.

The physiologic role of vitamin B₁₂ include:

- a. Conversion of methyl-malonyl-coenzyme A (CoA) to succinyl CoA by adenosyl cobalamin.
- b. Conversion of homocysteine to methionine.
- c. Synthesis of S-adenosyl-methionine.

3.1. Physiology of folate (pteroyl glutamic acid)

Folic acid, a composite molecule consists of pteridine, p-amino benzoic acid, and glutamic acid. Folate is available as polyglutamates in many foods like the green leafy vegetables, yeast, and liver. However, overcooking easily destroys the folate. Folate is absorbed as mono-glutamates in the upper jejunum. The daily requirement of folate is 150 µg and the body stores of folate are sufficient for 6 months. The major intracellular compounds are folate polyglutamates with attached additional glutamates. Folate is essential in many biochemical reactions like synthesis of purines, thymine, and deoxyribonucleic acid (DNA).

4. Etiology and pathogenesis

The principal causes of megaloblastic anemia in clinical practice are folate and cobalamin deficiency either directly or indirectly (see **Table 1**).

4.1. Major causes of cobalamin deficiency include

Dietary: Dietary cause of cobalamin deficiency is rare except in strict vegetarians who avoid taking meat, eggs, and dairy products.

Problems with cobalamin absorption: Atrophic gastritis and achlorhydria, which commonly occur in elderly people are the two conditions which are responsible for impaired release of cobalamins bound to food. Hence, cobalamin is not released from food for absorptive process.

I. Cobalamin deficiency	II. Folate deficiency	III. Drug-induced suppression of DNA synthesis	IV. Inborn errors
(i) Dietary deficiency	(i) Dietary deficiency	(i) Folate antagonists	(i) Defective transport of cobalamin
(ii) Deficiency of gastric IF Pernicious anemia or gastrectomy	(ii) Impaired absorption Sprue Extensive small bowel disease or resection Intestinal short circuits Anticonvulsants and oral contraceptives	(ii) Metabolic inhibitors of purine pyrimidine thymidylate synthesis Other inhibitors	(ii) Defective cobalamin utilization
(iii) Intestinal malabsorption Ileal resection or ileitis Familial selective cobalamin malabsorption Competitive parasites or infections Fish tapeworm Bacteria overgrowth in malformed small bowel	(iii) Increased requirement Pregnancy Hemolytic anemia Myeloproliferative and other hyperproliferative disorders	(iii) Alkylating agents D. Nitrous oxide (IV) Inborn errors	(iii) Defective folate metabolism (iv) Hereditary orotic aciduria E. Lesch-Nyhan syndrome
(iv) Increased Requirement		a. Defective transport of cobalamin b. Defective cobalamin utilization c. Defective folate metabolism d. Hereditary orotic aciduria e. Lesch-Nyhan syndrome	

Table 1. Pathogenetic classification of megaloblastic anemia [9].

Also, autoimmune destruction of gastric parietal cells may lead to failure of intrinsic factor production. This condition is called pernicious anemia. Pernicious anemia is recognized as the best-known cause of cobalamin deficiency. It is diagnosed in 1% of people older than 60 years and the incidence is slightly higher in women than in men.

Inhibition of intrinsic factor production can also be caused by H₂ antagonists.

The release of cobalamin from R-proteins can also be inhibited by the alkaline environment in the small intestine emanating from pancreatic insufficiency.

On the contrary, the acidic environment seen in conditions like Zollinger Ellison syndrome, also prevents binding of cobalamin to intrinsic factor hence leading to diminished binding to intrinsic factor and ultimate interference with cobalamin absorption.

The disorders of the terminal ileum, site of uptake of cobalamin-intrinsic factor complex, can cause cobalamin deficiency. Disorders that can possibly affect the terminal ileum include tropical sprue, inflammatory bowel disease, lymphoma, as well as ileal resection. Autoimmune destruction of the ileal receptor, cubilin, as found in Imerslund Grasbeck syndrome equally distrupts the uptake of cobalamin bound to intrinsic factor.

Also, bacteria colonization can occur in intestines deformed by strictures, surgical blind loops, scleroderma, inflammatory bowel disease, or amyloidosis blind loop syndrome can result to

cobalamin deficiency. In this condition, bacteria competes with the host for cobalamin for the uptake of cobalamin bound to intrinsic factor.

Fish tapeworm such as *Diphyllobothrium latum* infestation, which is common in places like Canada, Alaska, and the Baltic sea, feeds on cobalamin in the intestine thereby reducing the amount of cobalamin available for ingestion by the host.

Miscellaneous causes of cobalamin deficiency include exposure to nitrous oxide, which through oxidative inactivation of cobalamin causes megaloblastosis. Prolonged exposure to nitrous oxide can lead to severe mental and neurological disorders. Various medications like purine analogs (six mercaptopurine, six tioguanine), pyrimidine analogs (five fluorouracil and five azacytidine), and drugs that affect cobalamin metabolism like P-aminosalicylic acid, phenformin, and metformin that can cause cobalamin deficiency.

4.2. Major causes of folate deficiency

The main cause of loss of folate from food is poor food preparation through excessive dilution of food in water, through excessive heating, and subsequent inactivation of folate since folate is thermolabile. However, food fortification with folate and other vitamins are circumventing this problem in developed countries. This has to be aggressively promoted in many developing countries.

The storage of folate is for about 4 weeks after which folate deficiency sets in if folate intake is stopped. The daily requirement for adult is about 0.4 mg/day.

Folate deficiency occurs in situations where there is impaired absorption due to certain intestinal disorders like tropical sprue, nontropical sprue (celiac disease), amyloidosis, and inflammatory bowel disease.

Folate deficiency occurs in situations where there is increased physiologic demand for folate like chronic hemolytic states like sickle cell anemia, hereditary spherocytosis, and elliptocytosis; pregnancy, lactation, rapid growth, hyperalimentation, renal dialysis, where there is escalated loss of rapidly dividing cells like psoriasis and exfoliative dermatitis.

Also, medications such as phenytoin, metformin, phenobarbitone, dihydrofolate reductase, folate inhibitors like trimethoprim and pyrimethamine, methotrexate, sulphonamides, can cause folate deficiency.

Megaloblastic changes in human immunodeficiency virus (HIV) infection and myelodysplastic disorders are due to direct effect on deoxyribonucleic acid (DNA) in hemopoietic and other rapidly dividing cells.

4.3. Pathophysiology of megaloblastic anemia

The two vitamins, that is, folate and cobalamin act synergistically in generating the thymidyllic acid used for DNA synthesis. Therefore, in cobalamin deficiency, the megaloblastic arrest is actually caused by a deficit in folate utilization. As shown in **Figure 1** (activated methyl cycle), methionine is generated by transfer of methylene group from N5-methyl tetrahydrofolate

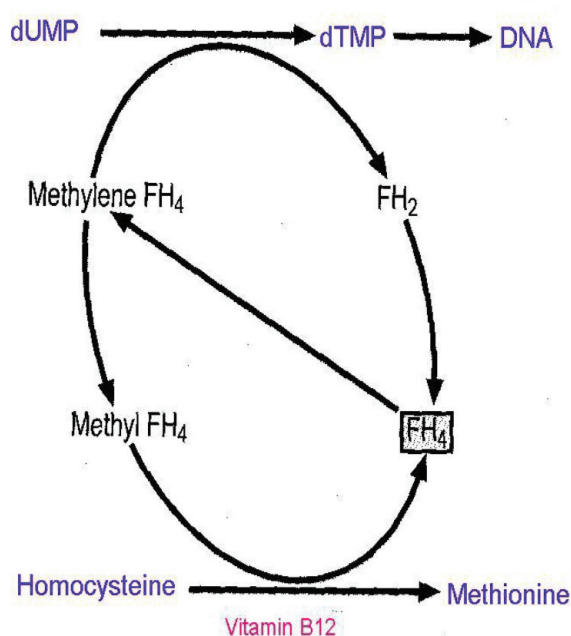


Figure 1. Activated methyl cycle.

(FH₄) to homocysteine using the enzyme methyl transferase (Methionine synthase). In this biochemical process, methylcobalamin is the factor that assists in methyl transfer as coenzyme form of cobalamin. This is why the morphological abnormalities emanating from either cobalamin or folate deficiency appear exactly alike.

5. Clinical features

Megaloblastic anemias, irrespective of the cause, share certain general features. The anemia develops slowly with little or no symptoms until the hematocrit is severely depressed and at this point, symptoms like weakness, palpitation, fatigue, light headedness, and shortness of breath occur. Severe pallor and light jaundice combine to produce a telltale lemon yellow skin. Slight differences occur in clinical symptoms and signs of megaloblastic anemia depending on whether it is caused by folate deficiency or by vitamin B₁₂ deficiency. In folate deficiency, main clinical features include anemic syndrome, pallor, icterus, hunter's syndrome, nail pigmentation, change of hair color (early graying), and splenomegaly in about 10–15% of patients. In addition to the above mentioned features, cobalamin deficiency manifests with neurological symptoms, which include loss of joint position sense in the second toes, loss of vibration sense in toes and fingers, paraesthesia, hypoesthesia, tingling sensation, gait abnormalities, loss of coordination, muscle weakness, spasticity, optic neuropathy, urinary and fecal incontinence, erectile dysfunction, dementia, memory loss. These neuropathies are symmetric and only affect lower extremities. Demonstrable signs include positive Romberg's sign, Babinsky reflex, thermittes's sign, spasticity, hyporeflexia, and clonus.

6. Laboratory features

The laboratory features of megaloblastic anemia revolve around the laboratory investigations and findings of vitamin B₁₂ and folate deficiencies (see **Figure 2**).

6.1. Blood cells

All the blood cells are affected. Erythrocytes vary markedly in size and shape (anisopoikilocytosis), some are large (twice in volume of normal red cells) and oval (egg shaped) (macro-ovalocytes) and in severe cases erythrocytes show basophilic stipplings and contain nuclear remnants (Howell-Jolly bodies, cabot rings). The morphologic changes are directly proportional to the severity of anemia. Circulating megaloblasts (i.e., nucleated red cells that failed to mature appropriately) are visible in circulation with hematocrit less than 20%. Anemia is typically macrocytic with a mean corpuscular volume (MCV) of 100–150 FL or more. However, the macrocytic appearance may be masked by coexisting iron deficiency, thalassaemia trait, and inflammation. It is noteworthy that slight macrocytosis is the earliest sign of megaloblastic anemia. Also mingled with macrocytes are fragmented red cells and tear drop poikilocytes. Reticulocytopenia (a reticulocyte count of <1%) is a frequent finding. This occurs because of inordinate impairment of erythropoiesis culminating in intramedullary destruction of megaloblasts and resultant reticulocytopenia. This is referred to as ineffective erythropoiesis (see **Figure 3**).

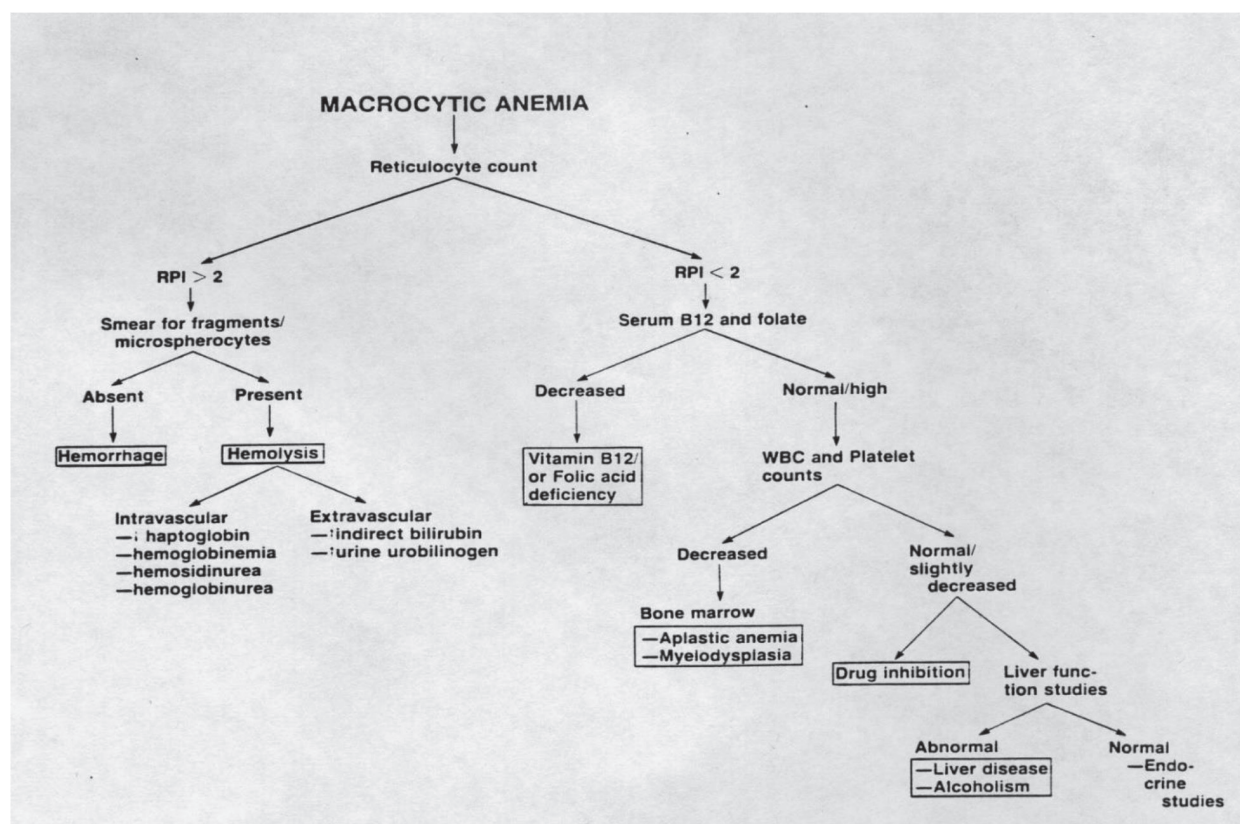


Figure 2. Algorithm for the investigation of macrocytic anemia.

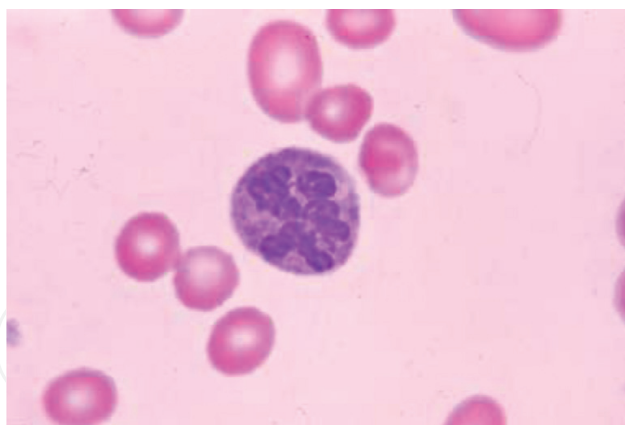


Figure 3. Cytologic abnormalities of megaloblastic anemia on blood smear.

As concerned about the leukocytes, there is a progressive reduction in white blood cells count but it rarely falls below 2000 cells/ μ L. Neutrophil hypersegmentation is another cardinal feature of megaloblastic anemia. In this case, neutrophil hypersegmentation is declared if neutrophils have more than the usual 3–5 nuclear segments/lobes. In other words, finding of a neutrophil having six or more nuclear segments or 5% of neutrophils have five or more lobes or in fact if most neutrophils have four or more lobes are strongly indicative of megaloblastic anemia. It is noteworthy that in nutritional megaloblastic anemias, hypersegmented neutrophils are an early sign of megaloblastosis.

Platelets vary widely in size and increased platelet distribution width (PDW) is the usual indicator. The complete blood count (CBC) often reveals anemia, leukopenia, and at times thrombocytopenia.

6.2. Serum vitamin B₁₂ and folate

These tests are known to be limited by their low sensitivity and specificity, and it has been shown that the normal lower limits for vitamin B₁₂ levels are not well defined [10]. Aside, these tests are expensive and not always available to the practicing clinician.

6.3. Serum B₁₂ levels

Previous studies showed that vitamin B₁₂ levels were found normal or elevated in myeloproliferative disorders, liver disease, congenital transcobalamin II deficiency, intestinal bacterial overgrowth, and antecedent administration of vitamin B₁₂ [11]

Falsely low vitamin B₁₂ levels with folate deficiency, pregnancy, use of oral contraceptives, congenital deficiency of serum haptocorrins, and multiple myeloma had been reported in Ref. [11].

6.4. Serum folate levels

Folic acid deficiency is rare where food fortification is the order of the day like in the US [12]. Although tissue stores may be normal, serum folate levels can decrease within a few days of

dietary folate restriction [11]. Thus, patients should fast prior to testing for serum folate levels, as serum folate levels increase with feeding. Mild degree of hemolysis can falsely display elevated serum folate levels because of high concentration of folate within the red blood cell (RBC) [11].

In spite of adequate tissue store of folate, certain conditions like pregnancy, use of certain anti-convulsant drugs and alcohol intake may also cause a decrease in serum levels of the vitamin. However, in vitamin B₁₂ deficiency serum folate levels tend to increase, probably because of impairment of the methionine synthase pathway and trapping of methyltetrahydrofolate, which happens to be the principal form of folate in the serum [13, 14].

6.5. Red blood cell (RBC) folate

In RBC, folate level is regarded as a more reliable source of determining tissue stores of folate. Unlike serum folate which is affected by dietary intake, RBC folate levels remain constant throughout the lifespan of the cell. However, assays for measuring RBC folate levels have also been fraught with unreliability [14–16]. Vitamin B₁₂ deficiency has been established to be a cause of low RBC folate levels [14, 17, 18]. It is estimated that approximately 60% of patients with pernicious anemia have low RBC folate levels, presumably because vitamin B₁₂ is necessary for normal transfer of methyl tetrahydrofolate from plasma to RBCs [13, 16, 19–21].

6.6. Bone marrow examination

The aspirated marrow is often hypercellular with striking imbalance in nuclear-cytoplasmic maturation often referred to as nuclear-cytoplasmic asynchrony. This asynchrony occurs because of progressive impaired DNA synthesis and nuclear derangements that accumulate with each cell division thereby slowing down nuclear replication and causes cumulative retardation with each step of maturation division. Therefore, the imbalance in cell growth becomes most apparent in matured hematopoietic cells. Sideroblasts, red cell precursors containing increased number of iron granules, are increased in proportion. Also, because of erythroid hyperplasia, the ratio of myeloid to erythroid precursors (M/E ratio) is reversed and may fall to 1:1 or even lower.

In severe cases, numerous giant pronormoblasts (promegaloblasts) having an unusually large number of mitotic figures are present. Macrophage iron content is often increased. Even with the attempt of masking megaloblastic anemia by the coexistence of microcytic anemia, a megaloblastic anemia will usually show hypersegmented neutrophils in the blood and giant metamyelocytes and bands in the marrow.

Substantial disintegration of erythroblasts occurs within the marrow sinuses sequel to undue prolonged detention of the erythroblasts with uncondensed nuclei wherein products of their disintegration are scavenged by macrophages. This process is referred to as ineffective erythropoiesis.

It is noteworthy that a megaloblastic anemia may be misdiagnosed as acute leukemia when megaloblastic anemia is very severe. In this case, the typical megaloblasts are obviously absent, and rather most cells available are bizarre megaloblastic pronormoblasts that dominate the marrow

because of lack of maturation of the erythroid series and hence raising the possibility of erythro-leukemia. On the contrary, a patient with just macrocytosis, no anemia, and without any cellular abnormality on the peripheral blood film may not require bone marrow examination.

The granulocytes and megakaryocytes are equally affected by the imbalance of cell growth in megaloblastic anemia. Myeloid cells are generally oversized but it is the presence of giant metamyelocytes and giant bound forms that are actually pathognomonic of megaloblastic anemia. There is also complex lobular hypersegmentation (pseudo hyperdiploidy) of megakaryocytes. There may be megakaryocyte fragments and giant platelets in circulation.

6.7. Serum concentrations of methylmalonic acid (MMA) and homocysteine

Several important metabolic pathways require the functions of cobalamin and folate as co-factors. The generation of methionine from homocysteine requires the co-factors of vitamin B₁₂ and folate. However, the production of succinyl CoA from L-methylmalonyl CoA requires only vitamin B₁₂. The generated succinyl CoA is involved in oxidative phosphorylation reactions within the cells. Therefore, early information regarding the cellular state of vitamin B₁₂ and folate are provided by these metabolites. The serum levels of these metabolites are helpful in distinguishing folate from vitamin B₁₂ deficiency [22, 23], whereas most patients with only folate deficiency have normal methylmalonic acid (MMA) or mildly elevated levels, patient with just vitamin B₁₂ deficiency do have significantly elevated level. It is noteworthy that almost 50% of patients with elevation of these metabolites do have normal serum vitamin B₁₂ levels. Hence, emphasizing the low sensitivity of serum vitamin B₁₂ levels, especially when there are implicating signs and symptoms.

Overall, measuring serum MMA and homocysteine levels are well established way of differentiating cobalamin deficiency from folate deficiency, whereas in cobalamin deficiency, both metabolites are elevated but anemic cobalamin deficient patient show more marked elevations [22, 24].

Nonanemia cobalamin deficient patients are better identified using MMA, which is far more sensitive than homocysteine, whereas in folate deficient patients there is marked elevation of homocysteine levels while serum levels of MMA are not elevated [22, 24].

Hence, measurement of serum level of these two metabolites provides a means of distinguishing cobalamin from folate deficiency as well as providing a reliable degree of accuracy in diagnosing these deficiency states [22, 24].

However, the sensitivity of identifying patients with cobalamin deficiency is masked by renal dysfunction leading to a falsely elevated serum MMA [24, 25]. Also, hereditary hyperhomocysteinemia, where elevated homocysteine may cause confusion in diagnosing folate deficiency. It is recommended that measurement of MMA should be undertaken only if the initial levels of vitamin B₁₂ and or homocysteine are abnormal.

6.8. Holotranscobalamin II (holoTC II)

When there is a discordance between vitamin B₁₂ levels and its metabolites, or even before measuring vitamin B₁₂, MMA, and/or homocysteine serum levels; holotranscobalamin II (holoTCII) is becoming an emerging marker that may be useful in establishing a diagnosis of

early vitamin B₁₂ deficiency. It is also a very useful marker in cases of renal failure or myeloproliferative diseases in which vitamin B₁₂ concentrations may be falsely elevated B₁₂ [24, 25]. Cobalamin is transported to cell membrane receptors by holotranscobalamin II and its serum concentration indirectly measures the amount of available vitamin B₁₂. It has been validated that holoTcII has greater sensitivity and specificity than serum level of vitamin although its routine use has not been recommended [23, 26].

Overall, the gold standard for the diagnosis of vitamin B₁₂ deficiency is yet to be established. Meanwhile, it is recommended that with low initial vitamin B₁₂ level (i.e., <150 ng/L) and in the setting of high clinical index of suspicion of vitamin B₁₂ deficiency, a repeat of serum level of the vitamin is suggested preferably along with MMA and homocysteine serum levels [27, 28]. However, in a case of unexplained macrocytic anemia, a complete diagnostic testing following a specific algorithm is necessary (Table 2) [29].

Laboratory studies and diagnostic ranges	Situations affecting results
Serum folate <2 ng/mL is diagnostic	Falsely low: Pregnancy, alcohol consumption, anti-seizure >4 ng/mL rules out deficiency drugs, temporarily (a few days) deficient diet (with normal)
Serum folate 2–4 ng/mL Falsely elevated	Requires quantification of methylmalonic acid, homocysteine and intra-erythrocyte folate. Single intake of folate-rich food.
Methyl-tetrahydrofolate [MTHF] and formyl-tetrahydrofolate (FTHF) <100–160 mg/L [Indicates deficiency]	Falsely low: Pregnancy, alcohol consumption, anti-seizure temporarily (a few days) deficient diet (with normal intra-erythrocyte folate). Compared with serum folate, less likely to alter due to transient variations such as dietary changes. Falsely elevated: Single intake of folate-rich food.
Serum cobalamin <200 pg/mL [diagnostic of deficiency] >300 pg/mL [rules out deficiency in 95% of cases]	Falsely low: Pregnancy, folate deficiency, HIV/Aids, anti-seizure drugs, multiply myeloma, hairy cell leukemia
200–300 pg/mL [indicates need to quantify methylmalonic acid and homocysteine]	Aplastic anemia, myelodysplastic syndromes, paroxysmal nocturnal hemoglobinuria, Gaucher's disease, oral contraceptives, Intra-individual variation: up to 23%, idiopathic origin, laboratory error.
Methylmalonic acid (MMA) Normal: 70–270 mmol/L	Falsely elevated: Kidney failure, methylmalonic acidemia. Intra-individual variation: up to 23%. Falsely low: Use of antibiotics. Usually elevated with comorbid cobalamin deficiency
Homocysteine Normal: 5–14 mmol/L	Falsely elevated: Hereditary hyperhomocysteinemia: Intra-individual variation: 17% changes in methyl-THFR, cystathionine beta-synthase, betaine usually elevated with comorbid cobalamin and folate synthesis. deficiency.
Elevated MMA and homocysteine:	Cobalamin deficiency (sensitivity: 94%, specificity: 99%). Normal MMA and homocysteine: rules out deficiency of both vitamins. Normal MMA and elevated homocysteine: folate deficiency (sensitivity: 86%, specificity: 99%).

Table 2. Specific laboratory investigations in suspected case of folate and cobalamin deficiency.

7. Differential diagnosis

Macrocytosis with MCV (not exceeding 110 FL) occurs in alcoholism, liver disease, hypothyroidism, aplastic anemia, myelodysplasia, pregnancy, and in certain disease states associated with a reticulocytosis (e.g., autoimmune hemolytic anemia).

8. Pernicious anemia (PA)

In pernicious anemia, the gastric parietal cells are destroyed by autoantibody and this results in failure or impaired production of intrinsic factor [30]. Frequently, both parietal cells and intrinsic factors are attacked by autoantibodies. The identification of parietal cell autoantibodies is more sensitive whereas the identification of intrinsic factor autoantibody is more specific.

Studies had shown that about 70% of patients with pernicious anemia will produce detectable levels of such autoantibodies. The clinical symptoms of pernicious anemia developed slowly as B₁₂ stores are sufficient for about 5 years before deficiency lead to the onset of clinical symptoms. Therefore, the full clinical picture of severe intramedullary hemolysis culminating in progressively severe chronic anemia, along with severe neurological symptoms with demyelination leading to weakness and paraplegia occurs only rarely. Treatment with parenteral vitamin B₁₂ will lead to a rapid increase of reticulocytes (within 48–72 h) and subsequent correction of anemia.

It is more common in males than in females and has an age peak around 60 year. In pernicious anemia, the gastric mucosa is atrophic and the secretion of intrinsic factor is defective. Inflammatory infiltrate of the gastric submucosa is the earliest gastric lesion in patients with PA. A type A gastritis involving the fundus and sparing the antrum is the typical finding in a patient with autoimmune pernicious anemia. The autoantibodies both to parietal cells and intrinsic factor are detectable both in the serum and gastric secretions. These autoantibodies particularly target the H⁺/K⁺-ATPase in the parietal cell resulting to gastric atrophy and achlorhydria. Studies had shown that parietal cell autoantibody is detectable in 90% of patients with pernicious anemia and also in 30% of first degree relatives who do not have pernicious anemia, and only about 2–8% of the normal population have low titer of these autoantibodies [31]. There are two types of anti-intrinsic factor autoantibodies. The type I autoantibodies blocks the binding of vitamin B₁₂ to intrinsic factor and type II auto antibody, seen in 35–40% of patients binds to different epitope of intrinsic factor [32].

A selective malabsorption of vitamin B₁₂ underlies the pathogenesis of autoimmune pernicious anemia. The eventual deficiency of vitamin B₁₂ resulted to megaloblastic anemia and ineffective erythropoiesis.

9. Treatment of cobalamin deficiency

To treat a case of megaloblastic anemia, all efforts should be applied to exclude the underlying cause. Drug-related causes, MDS, and others should be clearly excluded. Since

anemia of megaloblastosis insidiously develops, patients progressively adjust to the low hemoglobin and hence blood transfusion is not an option except only in patients with severe uncompensated and life threatening anemia.

Megaloblastic anemia with established cobalamin deficiency should be given intramuscular cobalamin of 1000 µg daily for 2 weeks or alternatively thrice weekly for 2 weeks for six doses and then weekly for another six doses until hematocrit returns to normal. It is given monthly for life in certain cases like pernicious anemia and in partial or total gastrectomy. Patients with neurological and mental impairment resulting from cobalamin deficiency deserve a very aggressive approach.

Oral cobalamin can be adopted when there is enough evidence that the absorptive capacity for cobalamin is intact. It is administered at 1000–2000 µg but a wide range of doses and schedules have been recommended. It is important to monitor closely for desired response since absorption can be variable and may be inadequate in some patients. Although intramuscular cobalamin is often preferable since it has the potential to bypass all abnormalities of cobalamin absorption. However, oral cobalamin is less expensive, better tolerated by patients, and preferable in patients with bleeding disorders like hemophilic patients in whom intramuscular injection should be avoided.

There is a need to apply multidisciplinary approach to the management of megaloblastic anemia. The hematologist hold a crucial position in making diagnosis and in management while the neurologists should be at hand to diagnose and manage potential neurological complications. The gastroenterologists are involved in ruling out the gastroenterological causes of the disease by doing both upper and lower endoscopy searching for diseases like atrophic gastritis, carcinoma of the stomach, and terminal ileitis. Also, pediatricians are needed in diagnosing and managing children with inborn errors and having megaloblastosis.

9.1. Folate therapy

In folate deficiency, a full hematologic response to physiologic doses of folate at 200 µg daily distinguishes it from cobalamin deficiency pharmacologic doses of folate at 5 mg daily is required to achieve full hematologic response [30]. This should not be recommended as a diagnostic test because neurologic problems may develop in cobalamin deficient patients treated with cobalamin alone. However, cobalamin may cause a partial response in folate deficiency [31].

Oral administration of folate is always the case except in difficult situation when parenteral administration may be indicated. At times folate rich diet may suffice. The dose of folate ranges between 1 and 5 mg daily but a higher dose is indicated in hemolytic conditions like sickle cell disease, hereditary elliptocytosis, hereditary spherocytosis, and in hyper-homocysteinemia. Food fortification with folate and supplementation are recommended to reduce the risk of pancreatic, cervical and colonic cancers, end stage renal disease and in elderly persons [32].

It is however noteworthy that administration of folate to individuals with Cobalamin deficiency increases the risk and frequency of cobalamin-induced neurological and neuropsychiatry disorders. Therefore, folate should not be instituted in patients with megaloblastic anemia when cobalamin deficiency has not been ruled out [33, 34].

The response to therapy should be closely monitored using complete blood count (CBC), reticulocyte count, lactate dehydrogenase (LDH) levels, indirect bilirubin, hemoglobin level, serum potassium and serum ferritin. It is expected that LDH and indirect bilirubin should fall rapidly with treatment while there is evident reticulocytosis within 3–5 days and it peaks between 4 and 10 days. The hemoglobin is expected to rise by 1 g/dL per week and should rise to normal level within 2 months. It is very important to closely monitor the serum potassium which falls with treatment and may result to death. Potassium supplementation should be given in case of hypokalemia using oral potassium supplement (slow K) Iron deficiency can occur because of escalated erythropoiesis and this may impede the rate of response. Iron therapy is equally necessary [33, 34].

A diagnostic therapeutic trial is allowed when the results of laboratory evaluation become ambiguous, a clinical trial of cobalamin therapy may be given. This is done only when cobalamin deficiency has been ruled out.

Particular attention should be placed on the followings:

Hypokalemia, that is, low serum potassium, can occur in a severe megaloblastic anemia on treatment because of ongoing rapid restoration of erythropoiesis in the bone marrow.

Only about 1% of ingested cobalamin is absorbed through the ileal mucosa when intrinsic factor is deficient. Therefore, a maintenance daily dose of 1 mg may be sufficient to maintain steady levels in patients not willing to receive regular injections.

It is also noteworthy that neurological symptoms may be irreversible or may respond slowly if folic acid was given without cobalamin in combined deficiency or while dealing with very severe and long standing cobalamin deficiency.

Author details

Olaniyi John Ayodele

Address all correspondence to: ayodeleolaniyi8@gmail.com

Department of Haematology, College of Medicine, University College Hospital, University of Ibadan, Ibadan, Nigeria

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